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Abstract: BACKGROUND: Skin test reactivity to hymenoptera venom and venom-specific IgE are important for diagnosing venom allergy and deciding on the appropriate allergen for venom immunotherapy (VIT). Longitudinal data on skin test reactivity during VIT and their correlation with venom-specific immunoglobulin (Ig)E and IgG are scarce. METHODS: We retrospectively analyzed shifts in skin test reactivity and serum levels of venom-specific IgE and IgG in patients allergic to hymenoptera venom before the initiation of VIT with ultrarush therapy and after 3 years of VIT. RESULTS: Fifty-four patients received ultrarush desensitization and subsequent VIT with wasp venom, 26 with honeybee venom, and 8 with both wasp and honeybee venom. Hymenoptera-specific skin test reactivity decreased during VIT in most patients, and became negative in 8% of the wasp-allergic patients and in 25% of the honeybee-allergic patients. Serum levels of venom-specific IgE positively correlated to skin test reactivity before VIT, but did not change significantly during VIT. IgG serum levels and the IgG/IgE ratio increased during VIT in most patients. A high IgG/IgE ratio correlated with low skin test reactivity after 3 years of VIT. CONCLUSIONS: The correlation between a high venom-specific IgG/IgE ratio and low skin test reactivity after VIT may be interesting for future investigations that assess its role as a potential marker for VIT efficacy.

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Skin Test Reactivity to Hymenoptera Venom after Venom Immunotherapy Correlates Inversely with the IgG/IgE Ratio

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Keywords
Hymenoptera venom allergy · Immunoglobulin E · Immunoglobulin G · Skin test · Venom immunotherapy

Abstract
Background: Skin test reactivity to hymenoptera venom and venom-specific IgE are important for diagnosing venom allergy and deciding on the appropriate allergen for venom immunotherapy (VIT). Longitudinal data on skin test reactivity during VIT and their correlation with venom-specific immunoglobulin (Ig)E and IgG are scarce. ***Methods:*** We retrospectively analyzed shifts in skin test reactivity and serum levels of venom-specific IgE and IgG in patients allergic to hymenoptera venom before the initiation of VIT with ultrarush therapy and after ≥3 years of VIT. ***Results:*** Fifty-four patients received ultrarush desensitization and subsequent VIT with wasp venom, 26 with honeybee venom, and 8 with both wasp and honeybee venom. Hymenoptera-specific skin test reactivity decreased during VIT in most patients, and became negative in 8% of the wasp-allergic patients and in 25% of the honeybee-allergic patients. Serum levels of venom-specific IgE positively correlated to skin test reactivity before VIT, but did not change significantly during VIT. IgG serum levels and the IgG/IgE ratio increased during VIT in most patients. A high IgG/IgE ratio correlated with low skin test reactivity after ≥3 years of VIT. ***Conclusions:*** The correlation between a high venom-specific IgG/IgE ratio and low skin test reactivity after VIT may be interesting for future investigations that assess its role as a potential marker for VIT efficacy.

Introduction

Wasp or honeybee venom allergy affects 0.3–7.5% of the general population [1]. Venom immunotherapy (VIT) is usually performed in individuals experiencing severe anaphylactic reactions [2]. VIT comprises venom injections during an updosing phase over several weeks and a maintenance phase for 3–5 years. Recently, ultrarush therapy protocols for updosing during only a few hours have been introduced to clinical routine. Ultrarush

This study was carried out at the Allergy Unit, Department of Dermatology, University and University Hospital of Zurich, Zurich, Switzerland

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therapy is safe, fast, provides optimal compliance, and is thus cost effective [3]. The accurate diagnosis of venom allergy and the identification of the causative insect are decisive in selecting the appropriate venom to use in VIT. The diagnostic workup comprises a thorough history, skin tests, and in vitro tests [4]. For skin testing, increasing concentrations of venom are injected intracutaneously until a positive reaction with a wheal and surrounding erythema (flare) is observed [5]. The skin test is considered the gold standard for the diagnosis of hymenoptera venom allergy, because it is sensitive to distinguishing sensitization to different types of venom, gives results within a few minutes, and may simulate the real-life situation of a sting better than in vitro tests [2, 4]. In vitro tests usually comprise the detection of immunoglobulin E (IgE) to whole venom and/or venom components such as the major wasp allergens Ves v 1 and Ves v 5 or the major bee allergen Api m 1, well as the detection of venom-specific IgG [6–8].

However, despite the regular use of skin tests and the measurement of venom-specific IgE and IgG before VIT, little is known about the longitudinal shifts of these parameters and the correlations between them during VIT. This is particularly true for patients who started VIT with ultrarush updosing [3]. We therefore carried out this study on wasp-allergic and/or honeybee venom-allergic patients undergoing VIT after ultrarush updosing, to assess (i) longitudinal shifts of skin test reactivity and venom-specific antibodies, and (ii) correlations between skin test reactivity and venom-specific antibodies before and after ≥3 years of VIT.

Methods

Patients

This was a retrospective, single-center study. We screened all medical files of patients undergoing ultrarush therapy and consecutive VIT with wasp or/and honeybee venom between 2010 and 2012 at the Allergy Unit, Department of Dermatology, University Hospital of Zurich. Inclusion criteria were (i) diagnosis of wasp and/or bee venom allergy that qualified for VIT, (ii) an age of ≥18 years, (iii) ultrarush therapy and VIT for a minimum of 3 years according the standard protocol of our department (see below), and (iv) data on venom skin test reactivity and venom-specific IgE and IgG from before ultrarush therapy versus after a minimum of 3 years of VIT. Exclusion criteria were (i) previous VIT with wasp and/or bee venom, (ii) the patient being a beekeeper, and (iii) systemic mastocytosis. The protocol for ultrarush sensitization and the maintenance phase of VIT was as follows. During ultrarush therapy, patients received increasing concentrations of total 111 μg of aqueous wasp or bee venom (Pharmalgen, ALK-Abello, Hørsholm, Denmark) into the dorsal upper arm within 2.5–3.5 h [3]. During the maintenance phase of VIT, patients received 100 μg of aluminium-adsorbed depot venom extract (Alutard SQ, ALK-Abello) every 4 weeks during the first year, every 5 weeks during the second year, and every 6 weeks during the third year after ultrarush therapy. Injections during the maintenance phase were either applied in our department or with a health care provider. Injection protocols of the maintenance phase were checked for violations of our standard protocol during the follow-up visit.

The study was conducted in accordance with the Declaration of Helsinki. For all patients, informed consent forms for the study were obtained in accordance with the Biobank Project (EK No. 647).

Clinical and Laboratory Parameters

In patients fulfilling the inclusion criteria, the following data were retrieved from the medical files: age; gender; a history of atopy defined as allergic rhinoconjunctivitis, and/or allergic asthma, or atopic dermatitis; the severity of the allergic reaction to the wasp or bee sting as graded by the criteria of Mueller [9]; the duration of VIT; the serum tryptase level; serum levels of IgE specific to whole wasp or bee venom (Ves v 1, Ves v 5, and Api m 1) before and after VIT; serum levels of wasp or bee venom-specific IgG before and after VIT; skin test reactivity to wasp or bee venom before and after VIT. For the diagnostic workup, laboratory tests and skin tests before VIT were performed >8 weeks after the culprit wasp or bee sting. At follow-up during or after VIT, laboratory tests and skin tests were performed ≥3 weeks after the last venom injection.

The levels of venom-specific IgE were determined by ImmunoCAP (Phadia, Thermo Fisher Scientific, Uppsala, Sweden), and those of venom-specific IgG were determined by a cellular allergy activation test followed by an enzyme-linked immunosorbent assay (Bühlmann Laboratories AG, Schönenbuch, Switzerland). For skin tests, 0.1 mL of increasing concentrations of aqueous extracts of wasp or bee venom (Pharmalgen) was injected intradermally into the lower forearms of patients. The 3 lowest venom concentrations (0.00001, 0.001, and 0.01 μg/mL), the negative and positive controls, were administered simultaneously. If these venom concentrations were negative, the 2 highest venom concentrations (0.1 and 1.0 μg/mL) were consecutively injected intradermally after 15 min each. All patients were allocated to 1 of 3 groups: group 1, patients receiving ultrarush therapy and VIT exclusively with wasp venom; group 2, patients receiving ultrarush therapy and VIT exclusively with honeybee venom; and group 3, patients receiving ultrarush therapy and VIT with both wasp and honeybee venom.

Statistics

Statistical analysis was performed with R software v3.2.3 [10]. The distribution of continuous data was tested for normality by means of the Kolmogorov-Smirnov and Lilliefors tests. Outliers of data points were visualized with Q-Q plots. We assumed a nonnormal distribution of continuous data among all groups and used nonparametric tests for further statistical analysis (the Wilcoxon rank test and the Kruskal-Wallis test). Frequencies of positive skin test results before versus after ≥3 years of VIT initiated by ultrarush were compared using the χ^2 test with the Yate continuity correction. The dilution series of 0.00001:1 μg/mL wasp or bee venom to determine skin test reactivity comprised 5 discrete data points, with a 6th data point for negative test results (equal to 10 μg/mL). This series of discrete data points was treated as continuous data for statistical analysis [11]. Continuous data (skin test reactivity

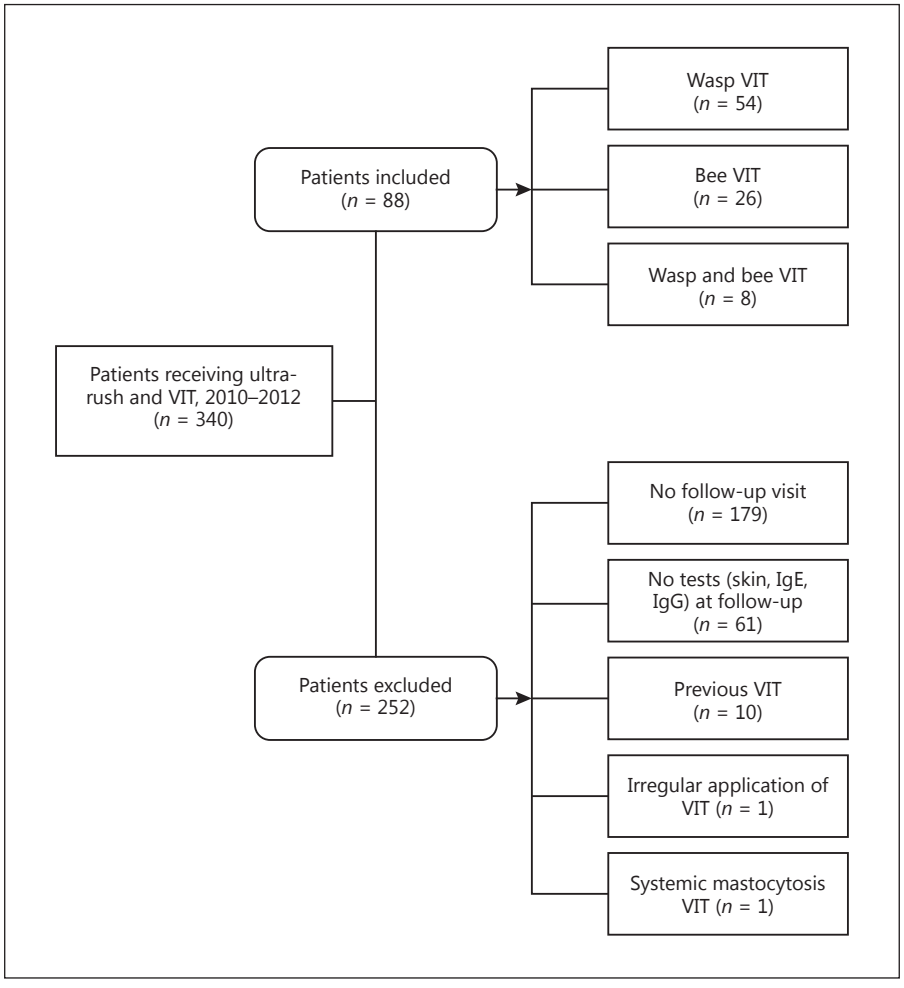

Fig. 1. Patient selection according to inclusion and exclusion criteria. VIT, venom immunotherapy.

and serum antibody titers) before versus after ≥3 years of VIT initiated by ultrarush desensitization were therefore compared using the Wilcoxon rank test. Clinical and laboratory metadata (gender, age, anaphylaxis grade, and serum tryptase level) were compared using the χ^2 test or the Kruskal-Wallis test. All p values were adjusted for multiple comparisons with the Benjamini-Hochberg correction [12]. The correlation between skin test reactivity and laboratory and clinical parameters was analyzed using the Spearman rho. Statistical significance for all tests was ascribed to 2-sided α level of the adjusted p values <0.05, and by 95% confidence intervals for continuous data.

Results

Patients

Between 2010 and 2012, a total of 340 patients received ultrarush therapy and VIT with wasp venom and/or honeybee venom in our department. Eighty-eight patients (25.8%) fulfilled the inclusion criteria and were further analyzed (Fig. 1). Fifty-four patients (61%) received ultrarush therapy and VIT with wasp venom ("wasp venom group," 27 females (50%); median age 44 years, interquartile range [IQR] 38–56 years), 26 patients (30%) with honeybee venom ("bee venom group," 13 females (50%); median age 46 years, IQR 24–52 years), and 8 patients (9%) with both wasp and honeybee venom ("wasp/bee venom group," 1 female (12.5%); median age 30 years, IQR 26–46 years). There was no statistically significant difference regarding the sex ($p = 0.11$) or age of patients ($p = 0.28$) in the 3 groups. All patients had a history of a systemic allergic reaction of grade II–IV after a wasp or bee sting (median grade III, IQR III–IV) according to Mueller [9]. The median time period between the initiation of VIT (including ultrarush therapy) and the follow-up control visit for laboratory analysis and skin test was 3 years (IQR 3–5 years) in all patient groups.

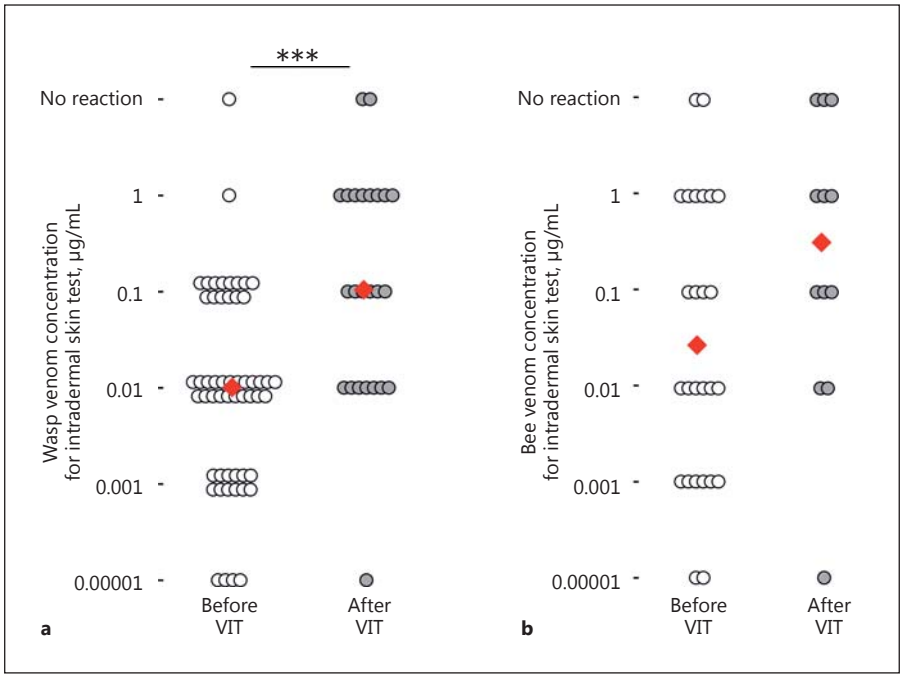

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Fig. 2. Concentration of the respective venom that was necessary to elicit a positive intradermal skin test before and after ≥3 years of venom immunotherapy (VIT) with wasp venom (**a**) and bee venom (**b**). The diamond indicates the median venom concentration among all patients. *** $p < 0.001$.

Longitudinal Shifts in Skin Test Reactivity

All patients received a skin test with both wasp and honeybee venom before ultrarush therapy. The majority of patients had a positive skin test before VIT. Only 1 patient in the wasp venom group (2%) and 2 patients in the bee venom group (8%) had a negative skin test before VIT. These 3 patients had an unambiguous history of allergic insect sting reactions and elevated venom-specific IgE to support the diagnosis of hymenoptera venom allergy.

After ≥3 years of VIT, a skin test was done on 24 patients in the wasp venom group (44%), 12 in the bee venom group (46%), and 5 in the wasp/bee venom group (63.5%). The skin test was performed at a median of 3 weeks after the previous venom injection (IQR 3–4 weeks). Although most patients remained positive at the skin test, we observed an average decrease of reactivity of 1 venom dilution step in the wasp venom and bee venom groups before versus after ≥3 years of VIT (Fig. 2). Only a few patients became negative at the skin test after ≥3 years of VIT (Table 1). This decrease was statistically significant in the wasp venom group ($p < 0.001$; 95% CI 1–2), but not in the bee venom group ($p = 0.2$; 95% CI –2 to 0.00002), presumably owing to the smaller number of patients in this group that had a skin test after ≥3 years of VIT ($n = 12$). We did not observe a significant shift in skin test reactivity before versus ≥3 years of VIT in the wasp/bee venom group, but the number of patients who received a skin test after ≥3 years of VIT in this group was too small ($n = 5$) for the statistical analysis.

Table 1. Dynamics of skin test reactivity after ≥3 years of venom immunotherapy

	Wasp venom group ($n = 24$)	Bee venom group ($n = 12$)	Wasp/bee venom group ($n = 5$)	
			wasp venom	bee venom
Decrease	17 (71)	5 (42)	2 (60)	3 (40)
Negative	2 (8)	3 (25)	0	0
Unchanged	3 (13)	3 (25)	1 (20)	2 (40)
Increase	2 (8)	1 (8)	1 (20)	1 (20)

Values are expressed as n (%). Patients in all 3 groups underwent skin testing before ultrarush therapy and after ≥3 years of venom immunotherapy.

Laboratory Parameters and Correlation with Skin Test Reactivity

Venom-specific IgE did not change significantly before versus after ≥3 years of VIT, in the wasp venom group (IgE to whole wasp venom, $p = 0.84$; Ves v 1, $p = 0.95$; Ves v 5, $p = 0.054$) or in the bee venom group (IgE

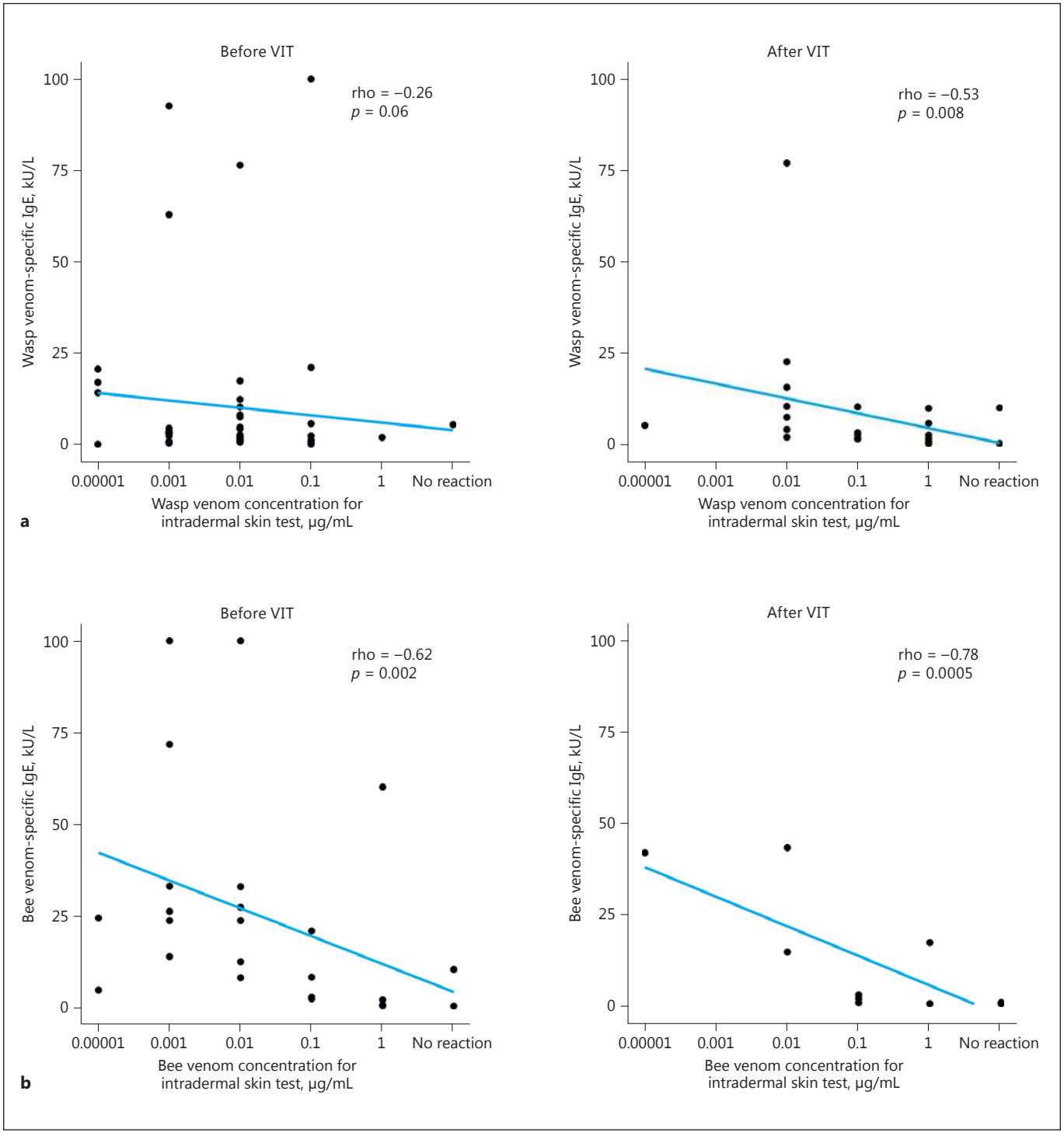

Fig. 3. Correlation between the venom concentration necessary to elicit a positive intradermal skin test and the serum levels of IgE to the respective whole venom before and after ≥3 years of venom immunotherapy (VIT) with wasp venom (**a**) and bee venom (**b**). The correlation was analyzed using the Spearman rho.

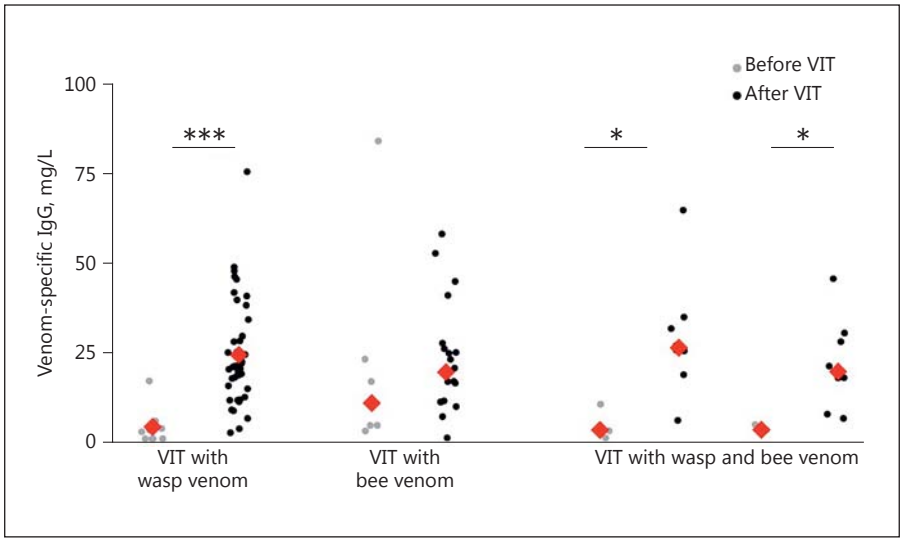

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Fig. 4. Serum levels of venom-specific IgG before and after ≥3 years of venom immunotherapy (VIT).* $p < 0.05$; *** $p < 0.001$.

to whole bee venom, $p = 1$; Api m 1, $p = 0.78$). Patients with higher serum IgE levels to the whole venom extract had a positive skin test to lower concentrations of the respective venom before and after ≥3 years of VIT. This correlation was generally weaker in the wasp venom group than in the bee venom group (Fig. 3). The number of patients in the wasp/bee venom group was too small for statistical analysis.

Serum levels of venom-specific IgG increased before versus after ≥3 years of VIT in all groups. This increase was statistically significant in the wasp venom-allergic patients of both the wasp venom group ($p < 0.001$) and the wasp/bee venom group ($p = 0.036$), and in the bee venom-allergic patients of the wasp/bee venom group ($p = 0.02$) but not the bee venom group ($p = 0.3$) (Fig. 4). Unlike with venom-specific IgE, serum levels of venom-specific IgG did not correlate with skin test reactivity to the respective venom at all (data not shown).

The ratio of venom-specific IgG/whole venom-specific IgE (IgG/IgE) significantly increased in the wasp venom group before versus after ≥3 years of VIT ($p < 0.01$). In the bee venom group, the IgG/IgE ratio increased in some patients after ≥3 years of VIT, but remained unchanged in others (Fig. 5). Patients with a higher venom-specific IgG/IgE ratio had lower skin test reactivity after ≥3 years of VIT than patients with a lower IgG/IgE ratio (Fig. 5).

We also investigated the correlation of the metadata, i.e., gender, age, anaphylaxis grade, presence of atopy, and serum tryptase level, with shifts in skin test reactivity and also venom-specific IgE and IgG, but no statistically significant correlation was observed.

Patients with Sting Challenges after VIT

Two bee-allergic patients underwent bee-sting provocation after the completion of VIT. This number was too small for statistical analysis. However, both patients tolerated the sting challenge without systemic or major local reactions, which indicates bee venom tolerance.

Discussion

This was a retrospective study of 88 patients undergoing ultrarush therapy and VIT with wasp and/or honeybee venom. We analyzed longitudinal shifts in skin test reactivity, serum levels of venom-specific IgE or IgG, and the correlation between these parameters. We found that skin test reactivity usually declined after ≥3 years of VIT, and became negative in 8 and 25% of wasp- and bee-allergic patients, respectively. This decline of skin test reactivity during VIT comprised a median 1 dilution of venom in both the wasp venom and bee venom groups. The statistically nonsignificant decline in the bee venom group was presumably due to the smaller number of patients that received a skin test at follow-up ($n = 12$) than there were in the wasp venom group ($n = 24$), and could therefore be a statistical phenomenon.

The decline of skin test reactivity observed in this study is in line with previous findings. In 2 studies on >580 wasp- or bee-allergic patients, skin test reactivity declined in about two-thirds of the wasp-allergic patients and in about half of the bee-allergic patients [13, 14]. There was a wide range of patients in which the skin test

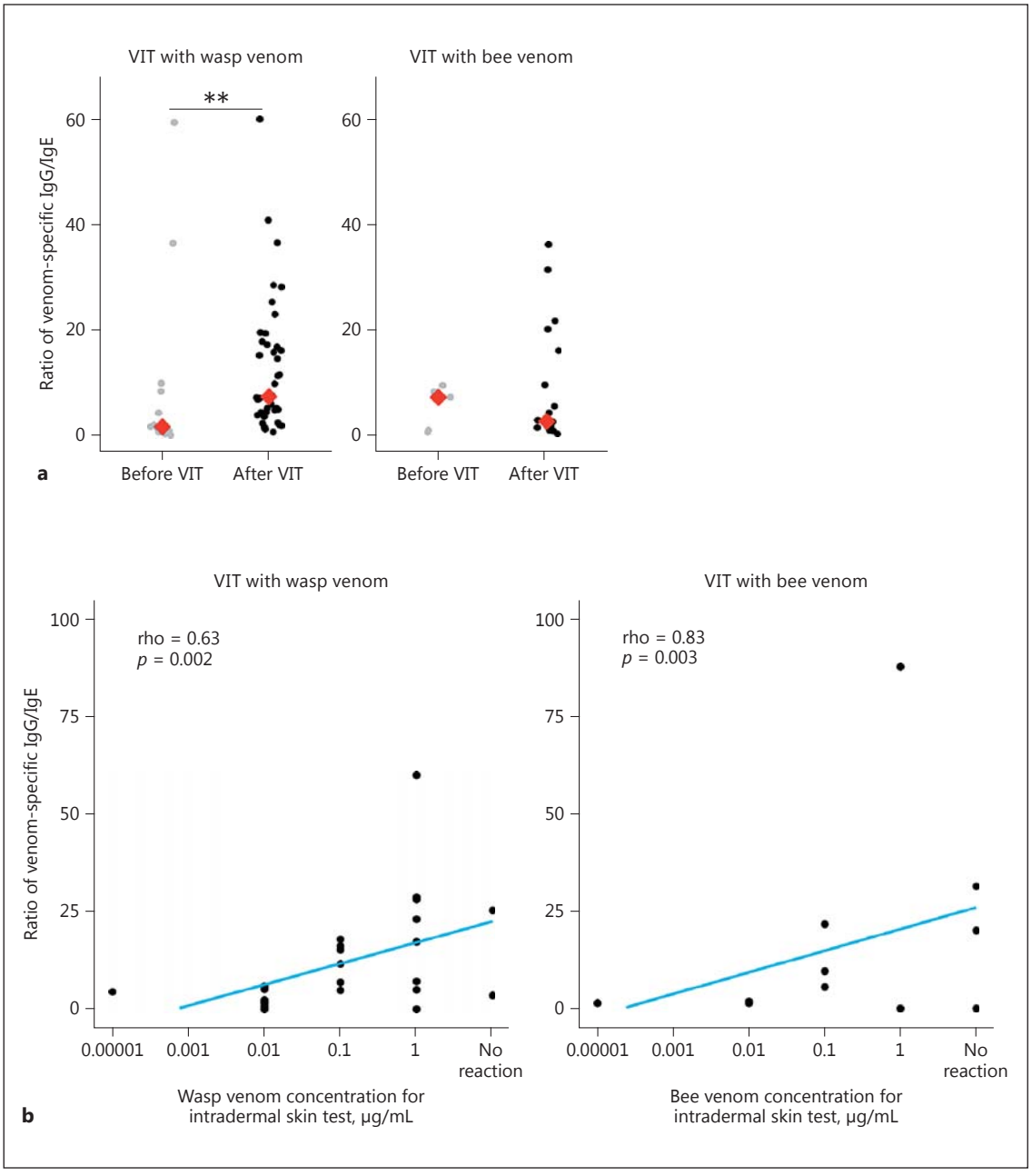

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Fig. 5. a Ratio of venom-specific IgG/whole venom-specific IgE before and after ≥3 years of venom immunotherapy (VIT). **b** Correlation between the venom concentration necessary to elicit a positive intradermal skin test and the ratio of venom-specific IgG/whole venom-specific IgE after ≥3 years of VIT. The correlation was analyzed using the Spearman rho. ** $p < 0.01$.

became negative after VIT, i.e., 6–27% of the wasp-allergic and 2.5–29% of the bee-allergic patients [13–17]. The rate of negative skin tests after VIT appears to be higher in studies that have applied an ultrarush protocol for venom updosing [16, 17]; this includes our study. It might therefore be speculated that the higher rate of skin test-negative patients after VIT in our study relates to the use of an ultrarush protocol for venom updosing. Another explanation could be the use of an aluminium-adsorbed depot extract during the maintenance phase of VIT in our

study. In contrast, previous studies with lower rates of negative skin test reactivity after VIT exclusively used an aqueous extract [13, 14]. However, until now, no differences have been noted in the efficacy of VIT when using these 2 venom extract types, as shown in a study on 27 patients undergoing VIT with a depot extract versus 18 patients undergoing VIT with an aqueous extract [18].

It remains unclear if a decrease in skin test reactivity after VIT indicates protection from allergic reactions at future allergen exposure. The wheal of a positive skin test reaction is caused by local, IgE-mediated mast cell activation, indicates allergen sensitization, and may also hint at a possibly systemic mast cell reaction after allergen exposure, e.g., after a wasp or bee sting [19]. Therefore, some authors attribute a decreased reactivity in these skin tests as a possible indicator of the lower susceptibility of mast cells to an allergen, and hence a hint of an efficacious VIT. They suggest skin test negativity as the goal of a successful VIT [13, 20, 21]. However, only a minority of patients achieve negativity in the skin test after VIT [13, 14, 17], and a decrease in skin test reactivity may not necessarily indicate tolerance of restings after VIT, e.g., at sting challenges [13, 15, 20].

Another possible parameter to assess venom tolerance is a sting challenge, but a tolerated sting challenge does not necessarily predict tolerance of future sting in an individual patient [1, 4, 22–24]. Moreover, up to 17% of patients experience systemic reactions during these sting challenges, even after 1–5 years of VIT [25, 26]. These reactions can be near-fatal or even fatal, experienced in our department (unpubl. data) and also reported by other study groups [20, 27]. This corroborates the need for a reliable and safe parameter to assess VIT efficacy. To support the search for a new parameter that fulfills these requirements, we were interested to further assess possible shifts in laboratory parameters before versus after VIT, and to correlate these shifts with skin test reactivity.

We did not observe a significant shift of venom-specific IgE, but there was an increase in venom-specific IgG before versus after ≥3 years of VIT. Some previous studies described the opposite, namely, a significant decrease in venom-specific IgE but unchanged serum levels of venom-specific IgG. This may be attributed to (i) the use of different detection methods such as a radioallergosorbent assay for IgE and another ELISA for IgG [13] compared to the ImmunoCAP and CAST/ELISA used in our study; (ii) a shorter duration of VIT, like 1 year [14] compared to the ≥3 years in our study; or (iii) the use of different immunotherapy protocols. Increased IgG antibody levels during VIT have been described previously, e.g., in a case report on a beekeeper's wife that changed VIT from whole bee extract to pure bee venom [28], and in a study on 40 patients with yellow-jacket venom allergy [29]. However, these studies either described a single patient [28], or the IgG levels were measured already after a few weeks after the onset of VIT and by a method different from ours [29]. A more recent study by Schiavino et al. [17] applied an ultrarush protocol similar to ours, and it described an increase of IgG but unchanged IgE during VIT, as described in our study. It therefore remains possible that the venom updosing with ultrarush therapy modulates the immune response to an allergen differently from a conventional updosing protocol [30], but the exact mechanisms remain to be elucidated. Ambiguous results regarding shifts of IgG before and after VIT could also be explained by the detection of total IgG versus IgG_4 in different studies. In our study, we measured total IgG rather than IgG_4 only, because IgG_1 also has important protective effects after VIT by blocking IgE-mediated reactions via different mechanisms [31]. For example, IgG can directly bind to an allergen and therefore block the allergen recognition by IgE. IgG also binds the inhibitory FcεRIIb receptor on mast cells and abrogates mast cell activation by IgE [32]. However, a correlation between increased IgG after VIT and protection from allergic reactions after future stings has not yet been proven [33]. This might be due to the possibility that not all IgGs bind to epitopes on allergens that are recognized by IgE, hence allergen recognition by IgE is not totally blocked despite high IgG levels [34]. Therefore, the IgG/IgE ratio might be a better indicator for the protection against allergic reactions [35]. We found an increase of the venom-specific IgG/IgE ratio in all wasp-allergic and in some bee-allergic patients before versus after ≥3 years of VIT, which is in line with previous studies [21, 36]. We also investigated the correlation between skin test reactivity and IgG/IgE ratio after 3 years VIT which, to our knowledge, has not yet been published. We found that wasp- and bee-allergic patients with a high IgG/IgE ratio have low skin test reactivity after ≥3 years of VIT. But it is unclear if a high IgG/IgE ratio after VIT indeed indicates protection from allergic reactions to wasp or bee restings. Early studies on 176 wasp- or bee-allergic patients did not observe a difference in the outcome of diagnostic sting challenge, depending on the serum levels of venom-specific IgE and IgG or the IgG/IgE ratio [37, 38]. Notably, these were diagnostic studies and they measured the serologic parameters before VIT for diagnostic purposes but not after VIT, as in our study.

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Disclosure Statement

The authors declare no conflict of interest for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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